



TABLE 1: Virus Employed in Biological Assay

Vesicular stomatitis virus-glycoprotein ("VSV-g") pseudotyped HIV-1 luciferase reporter virus ("HIV-1 pseudovirions") was used in this assay. The virus was harvested from Human Embryonic Kidney (HEK) 293T producer cells ("HEK293T") following a triple transient transfection (CaP, Clontech) of the three plasmid HIV-1 lentiviral vector system comprised of the VSV-g envelope expression plasmid, packaging construct (delta psi) and the HIV-1 LTR:Luc plasmid. The VSV-g envelope expression plasmid generates the pseudovirus receptor that permits a broad tropism and mediates entry into the HEK293T target cells. The delta psi packaging construct supplies all of the structural and regulatory gene products needed to generate the pseudovirus. The viral vector RNA synthesized from the HIV-1 LTR:Luc plasmid possesses the *cis* RNA packaging signal (psi sequence) in addition to the luciferase reporter gene and the HIV-1 LTR. The supernatants of transfected producer cells contain HIV-1 pseudovirions carrying only the luciferase gene in the viral genome. Upon transduction of the target 293T cells, the viral genomic RNA will undergo reverse transcription, nuclear translocation, integration, and transcription of the integrated luciferase gene driven by the PGK (phosphoglycerate kinase promoter). Luciferase activity using Bright-Glo reagent (Promega) substrate was measured 48 hours post-infection using a CLIPR™ plate reader (Molecular Devices) to determine EC50 values.



TABLE 2: Assay Protocol

Activity against replication-defective HIV reporter virus (single-cycle infection assay) in 293T target cells:

HEK 293T cells are routinely cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS, 1X Pen/Strep/Glutamine. The protocol is as follows:

1. 293T cells are seeded in the 1536-well format at 700 cells/well (5 μ L volume) using an AquaMax (Molecular Devices) liquid dispenser.
2. Cells are cultured at 37°C under 5% CO₂ for 24 hours.
3. 50 nL of each compound (serially diluted in DMSO) are transferred using the PinTool (GNF).
4. After a 1 hour, 37°C incubation, HIV reporter virus is transferred to the cells using the AquaMax in a volume of 2 μ L corresponding to a multiplicity of infection (MOI) of approximately 1.0.
5. The treated and infected cells are cultured for an additional 48 hours at 37°C.
6. Luciferase activity is monitored by addition of Bright-Glo (Promega, Cat.# E263B and E264B) luciferase reagent (5 μ L /well, AquaMax) followed by plate reading on the CLIPR™ apparatus (Molecular Devices) using a 20 second shuttle speed.

Cytotoxicity Assay (in parallel with all infection inhibition assays)

1. 293T cells are seeded in the 1536-well format at 700 cells/well (5 μ L volume) using an AquaMax (Molecular Devices) liquid dispenser.
2. Cells are cultured at 37°C under 5% CO₂ for 24 hours.
3. 50 nL of each compound (serially diluted in DMSO) are transferred using the PinTool (GNF).
4. The treated and uninfected cells are cultured for an additional 48 hours at 37°C.
5. Cell viability is assessed by addition of 1 μ L of Alamar Blue (Promega, Cat.# 00-100) diluted 1:1 in DMEM.
6. Cells are further incubated for 4 hours at room temperature and subsequent fluorescence intensity is read using an Acquest (TREK systems) with a 50/50 beam splitter.



TABLE 3A: Physical and Biological Data for the Quinolone Compounds of U.S. Pat. App. No. 10/690,738

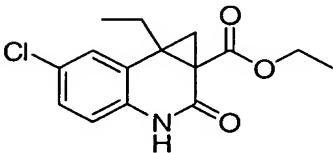
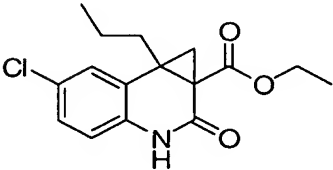
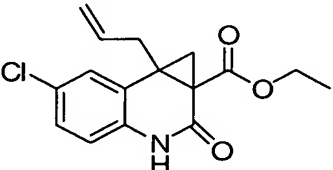
Compound Number	Structure	Physical Data ¹ H NMR 400 MHz (DMSO- <i>d</i> ₆) and/or MS (<i>m/z</i>)	EC ₅₀ (μM) (293 T)
1		MS <i>m/z</i> 294.1 (<i>M</i> + 1).	5.03
2		MS <i>m/z</i> 308.1 (<i>M</i> + 1).	0.045
3		¹ H NMR 400 MHz (CDCl ₃) δ 8.96 (bs, 1H), 7.25 (d, 1H), 6.98 (dd, 1H), 6.61 (d, 1H), 5.53 (m, 1H), 4.95-4.89 (m, 2H), 4.16-4.05 (m, 2H), 2.98(dd, 1H), 2.05 (d, 1H), 2.01 (dd, 1H), 1.12 (t, 3H), 0.98 (d, 1H); MS <i>m/z</i> 306.2 (<i>M</i> + 1).	0.37

TABLE 3B

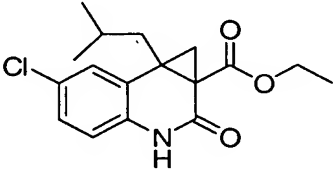
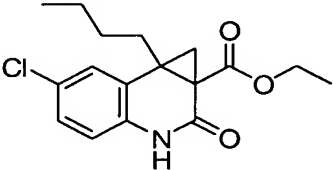
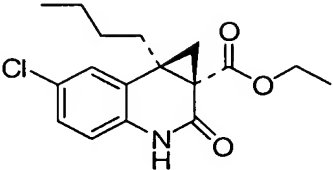
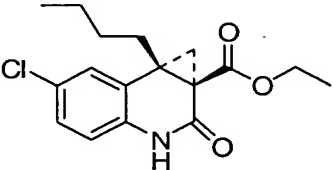
Compound Number	Structure	Physical Data ¹ H NMR 400 MHz (DMSO- <i>d</i> ₆) and/or MS (<i>m/z</i>)	EC ₅₀ (μM) (293 T)
4		MS <i>m/z</i> 322.1 (<i>M</i> + 1).	0.057
5		¹ H NMR 400 MHz (Acetone- <i>d</i> ₆) δ 8.99 (bs, 1H), 7.35 (d, 1H), 7.14 (dd, 1H), 6.76 (d, 1H), 4.30 (q, 2H), 2.45 (m, 1H), 2.16 (d, 1H), 1.45 (m, 1H), 1.30-1.20 (m, 7H), 1.07 (d, 1H), 0.86 (t, 3H); MS <i>m/z</i> 322.1 (<i>M</i> + 1).	0.003
6		¹ H NMR 400 MHz (Acetone- <i>d</i> ₆) δ 8.99 (bs, 1H), 7.35 (d, 1H), 7.14 (dd, 1H), 6.76 (d, 1H), 4.30 (q, 2H), 2.45 (m, 1H), 2.16 (d, 1H), 1.45 (m, 1H), 1.30-1.20 (m, 7H), 1.07 (d, 1H), 0.86 (t, 3H); MS <i>m/z</i> 322.1 (<i>M</i> + 1).	0.003
7		¹ H NMR 400 MHz (Acetone- <i>d</i> ₆) δ 8.99 (bs, 1H), 7.35 (d, 1H), 7.14 (dd, 1H), 6.76 (d, 1H), 4.30 (q, 2H), 2.45 (m, 1H), 2.16 (d, 1H), 1.45 (m, 1H), 1.30-1.20 (m, 7H), 1.07 (d, 1H), 0.86 (t, 3H); MS <i>m/z</i> 322.1 (<i>M</i> + 1).	8.75

TABLE 3C

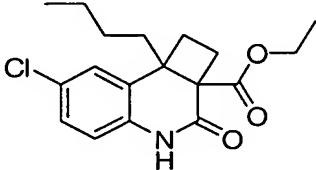
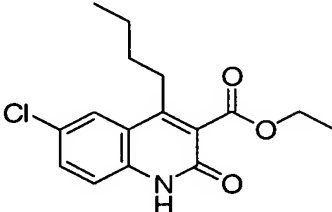
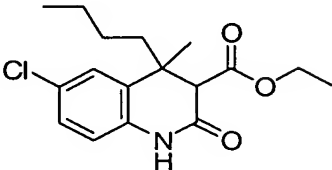
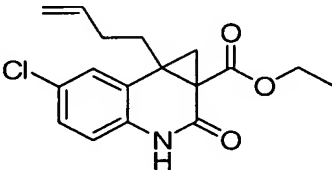
Compound Number	Structure	Physical Data ¹ H NMR 400 MHz (DMSO- <i>d</i> ₆) and/or MS (m/z)	EC ₅₀ (μM) (293 T)
8		¹ H NMR 400 MHz (CDCl ₃) δ 9.97 (s, 1H), 7.11–6.99 (m, 2H), 6.65 (dd, 1H), 4.27 (m, 1H), 4.15 (m, 1H), 2.75 (m, 1H), 2.35–2.15 (m, 2H), 2.09 (m, 1H), 1.79–1.55 (m, 2H), 1.23 (t, 3H), 1.22–1.05 (m, 4H), 0.72 (t, 3H); MS <i>m/z</i> 336.2 (M + 1).	0.102
9		¹ H NMR 400 MHz (CDCl ₃) δ 7.90 (bs, 1H), 7.48 (dd, 1H), 7.33 (d, 1H), 7.23 (d, 1H), 4.48 (q, 2H), 2.82–2.75 (m, 2H), 1.69–1.51 (m, 4H), 1.50–1.41 (m, 3H), 0.97 (t, 3H).	0.166
10		MS <i>m/z</i> 324.1 (M + 1).	1.398
11		MS <i>m/z</i> 320.1 (M + 1).	0.003

TABLE 3D

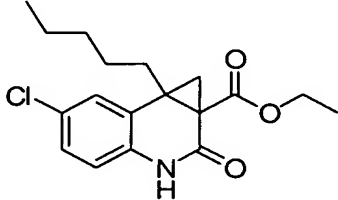
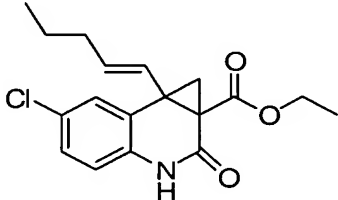
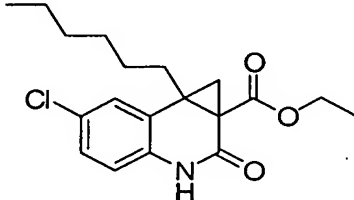
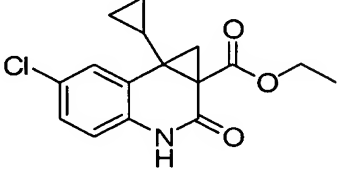
Compound Number	Structure	Physical Data ¹ H NMR 400 MHz (DMSO- <i>d</i> ₆) and/or MS (<i>m/z</i>)	EC ₅₀ (μM) (293 T)
12		MS <i>m/z</i> 336.1 (M + 1).	0.003
13		¹ H NMR 400 MHz (CDCl ₃) δ 8.24 (s, 1H), 7.27 (d, 1H), 7.08 (dd, 1H), 6.64 (d, 1H), 5.76 (m, 1H), 5.50 (d, 1H), 4.17-4.07 (m, 2H), 2.54 (d, 1H), 2.03 (m, 2H), 1.37 (m, 2H), 1.17 (t, 3H), 1.07 (d, 1H), 0.87 (t, 3H); MS <i>m/z</i> 334.25 (M + 1).	0.11
14		MS <i>m/z</i> 350.1 (M + 1).	0.025
15		MS <i>m/z</i> 306.1 (M + 1).	16.89

TABLE 3E

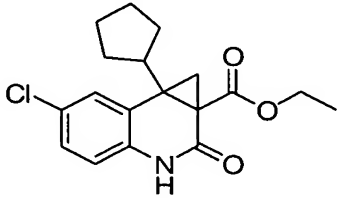
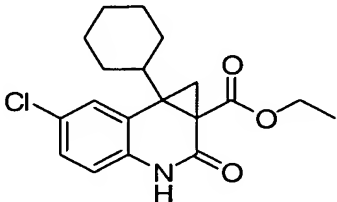
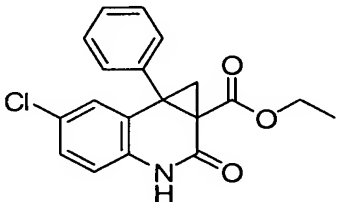
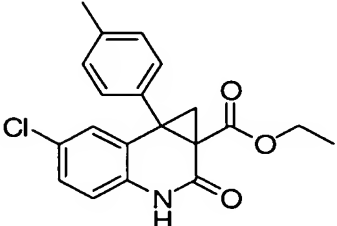
Compound Number	Structure	Physical Data ¹ H NMR 400 MHz (DMSO- <i>d</i> ₆) and/or MS (<i>m/z</i>)	EC ₅₀ (μM) (293 T)
16		MS <i>m/z</i> 334.1 (M + 1).	3.24
17		MS <i>m/z</i> 348.1 (M + 1).	1.46
18		¹ H NMR 400 MHz (Acetone- <i>d</i> ₆) δ 9.67 (bs, 1H), 7.58 (dd, 1H), 7.44 (m, 1H), 7.37-7.35 (m, 2H), 7.24-7.19 (m, 2H), 7.08 (d, 1H), 6.65 (d, 1H), 3.78 (q, 2H), 2.83 (d, 1H), 2.04 (d, 1H), 0.90 (t, 3H).	NA
19		MS <i>m/z</i> 356.10 (M + 1).	2.89

TABLE 3F

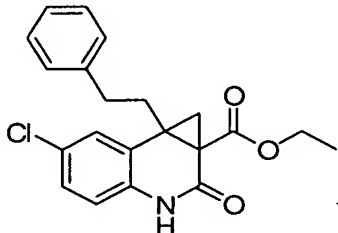
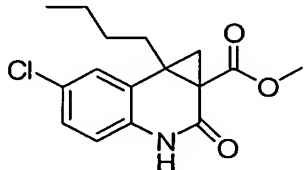
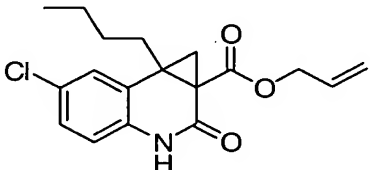
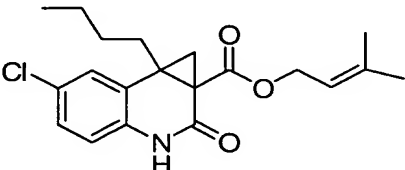
Compound Number	Structure	Physical Data ¹ H NMR 400 MHz (DMSO- <i>d</i> ₆) and/or MS (<i>m/z</i>)	EC ₅₀ (μM) (293 T)
20		MS <i>m/z</i> 370.1 (M + 1).	0.221
21		¹ H NMR 400 MHz (CDCl ₃) δ 7.78 (s, 1H), 7.30 (d, 1H), 7.08 (dd, 1H), 6.60 (d, 1H), 3.75 (s, 3H), 2.36 (m, 1H), 2.12 (d, 1H), 1.40 (m, 1H), 1.24-1.13 (m, 5H), 0.80 (t, 3H); MS <i>m/z</i> 308.2 (M + 1).	0.003
22		¹ H NMR 400 MHz (CDCl ₃) δ 9.25 (s, 1H), 7.29 (d, 1H), 7.07 (dd, 1H), 6.71 (d, 1H), 5.86 (m, 1H), 5.37 (dd, 1H), 5.20 (dd, 1H), 4.70 (dd, 1H), 4.64 (dd, 1H), 2.41 (m, 1H), 2.11 (d, 1H), 1.35 (m, 1H), 1.21-1.02 (m, 4H), 1.03 (d, 1H), 0.78 (t, 3H); MS <i>m/z</i> 334.2 (M + 1).	0.003
23		¹ H NMR 400 MHz (CDCl ₃) δ 9.29 (bs, 1H), 7.28 (d, 1H), 7.06 (dd, 1H), 6.72 (d, 1H), 5.29 (m, 1H), 4.71 (dd, 1H), 4.64 (dd, 1H), 2.38 (m, 1H), 2.10 (d, 1H), 1.67 (s, 3H), 1.65 (s, 3H), 1.32 (m, 1H), 1.20-1.14 (m, 4H), 1.00 (d, 1H), 0.79 (t, 3H); MS <i>m/z</i> 362.9 (M + 1).	2.36

TABLE 3G

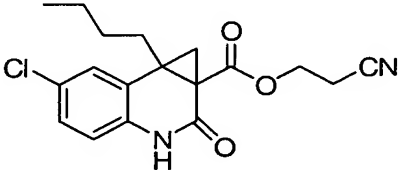
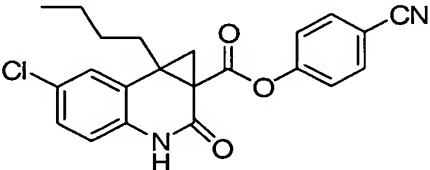
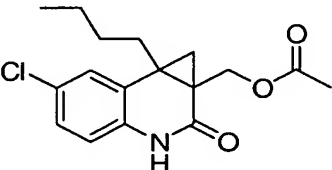
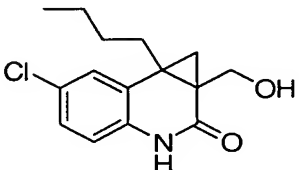
Compound Number	Structure	Physical Data ¹ H NMR 400 MHz (DMSO- <i>d</i> ₆) and/or MS (<i>m/z</i>)	EC ₅₀ (μM) (293 T)
24		¹ H NMR 400 MHz (CDCl ₃) δ 7.79 (s, 1H), 7.30 (d, 1H), 7.10 (dd, 1H), 6.62 (d, 1H), 4.38 (m, 1H), 4.30 (m, 1H), 2.69 (m, 1H), 2.43 (m, 1H), 2.12 (d, 1H), 1.41 (m, 1H), 1.15-1.10 (m, 4H), 1.08 (d, 1H), 0.97 (m, 1H), 0.81 (t, 3H).	0.133
25		¹ H NMR 400 MHz (CDCl ₃) δ 7.97 (s, 1H), 7.65 (dd, 2H), 7.34 (d, 1H), 7.23 (dd, 2H), 7.12 (dd, 1H), 6.65 (d, 1H), 2.50 (m, 1H), 2.19 (d, 1H), 1.32-1.23 (m, 5H), 1.18 (d, 1H), 0.81 (t, 3H); MS <i>m/z</i> 395.4 (M + 1).	1.09
26		¹ H NMR 400 MHz (CDCl ₃) δ 8.34 (s, 1H), 7.31 (d, 1H), 7.07 (dd, 1H), 6.64 (d, 1H), 4.98 (d, 1H), 4.03 (d, 1H), 2.48 (m, 1H), 1.98 (s, 3H), 1.41 (m, 1H), 1.35 (d, 1H), 1.30-1.20 (m, 4H), 0.90 (d, 1H), 0.82 (t, 3H); MS <i>m/z</i> 322.2 (M + 1).	0.037
27		MS <i>m/z</i> 280.2 (M + 1).	0.74

TABLE 3H

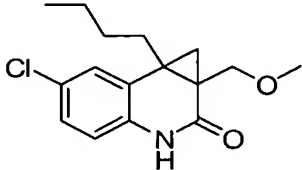
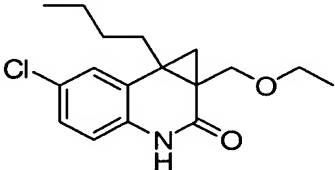
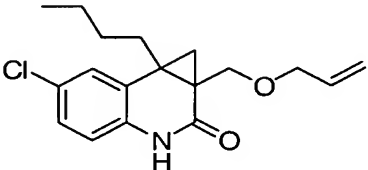
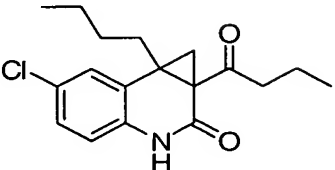
Compound Number	Structure	Physical Data ¹ H NMR 400 MHz (DMSO- <i>d</i> ₆) and/or MS (<i>m/z</i>)	EC ₅₀ (μM) (293 T)
28		¹ H NMR 400 MHz (CDCl ₃) δ 8.14 (s, 1H), 7.30 (d, 1H), 7.03 (dd, 1H), 6.59 (d, 1H), 4.23 (d, 1H), 3.35 (d, 1H), 3.31 (s, 3H), 2.39 (m 1H), 1.39-1.27 (m, 6H), 0.85 (t, 3H), 0.80 (d, 1H); MS <i>m/z</i> 294.2 (M + 1).	0.045
29		¹ H NMR 400 MHz (CDCl ₃) δ 8.01 (s, 1H), 7.30 (d, 1H), 7.02 (dd, 1H), 6.57 (d, 1H), 4.30 (d, 1H), 3.52-3.39 (m, 3H), 2.40 (m, 1H), 1.45-1.15 (m, 6H), 1.12 (t, 3H), 0.85 (t, 3H), 0.79 (d, 1H); MS <i>m/z</i> 308.3 (M + 1).	0.065
30		¹ H NMR 400 MHz (CDCl ₃) δ 7.87 (s, 1H), 7.30 (d, 1H), 7.02 (dd, 1H), 6.50 (d, 1H), 5.83 (m, 1H), 5.20 (dd, 1H), 5.11 (dd, 1H), 4.31 (d, 1H), 4.04-3.90 (m, 1H), 3.43 (d, 1H), 2.40 (m, 1H), 1.35-1.18 (m, 7H), 0.84 (t, 3H), 0.77 (d, 1H); MS <i>m/z</i> 320.2 (M + 1).	14.68
31		MS <i>m/z</i> 320.2 (M + 1).	0.265

TABLE 3I

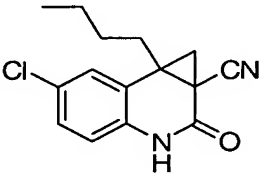
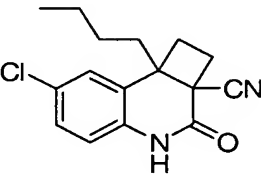
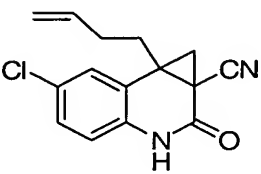
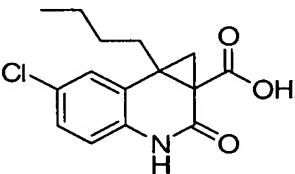
Compound Number	Structure	Physical Data ¹ H NMR 400 MHz (DMSO- <i>d</i> ₆) and/or MS (<i>m/z</i>)	EC ₅₀ (μM) (293 T)
32		¹ H NMR 400 MHz (CDCl ₃) δ 9.14 (br s, 1H), 7.32 (d, 1H), 7.18 (dd, 1H), 6.79 (d, 1H), 2.66 (m, 1H), 2.04 (d, 1H), 1.48-1.32 (m, 6H), 0.86 (t, 3H); MS <i>m/z</i> 275.2 (M + 1).	0.197
33		¹ H NMR 400 MHz (CDCl ₃) δ 9.63 (s, 1H), 7.21 (d, 1H), 7.15 (d, 1H), 6.81 (d, 1H), 2.68 (m, 1H), 2.57 (d, 1H), 2.49 (m, 1H), 2.21 (m, 1H), 2.13 (m, 1H), 1.79 (m, 1H), 1.35-1.07 (m, 3H), 0.85-0.65 (m, 4H); MS <i>m/z</i> 289.2 (M + 1).	6.29
34		MS <i>m/z</i> 273.1 (M + 1).	0.98
35		MS <i>m/z</i> 294.3 (M + 1).	5.89

TABLE 3J

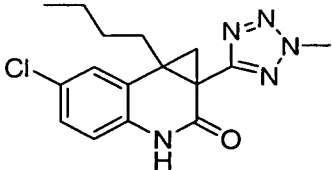
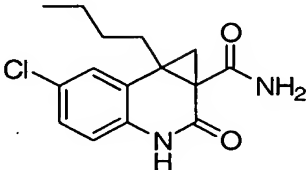
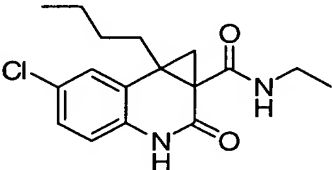
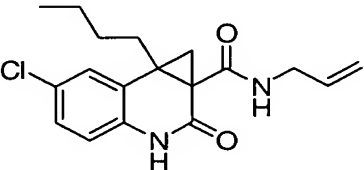
Compound Number	Structure	Physical Data ¹ H NMR 400 MHz (DMSO- <i>d</i> ₆) and/or MS (<i>m/z</i>)	EC ₅₀ (μM) (293 T)
36		¹ H NMR 400 MHz (CDCl ₃) δ 8.93 (s, 1H), 7.27 (d, 1H), 7.07 (dd, 1H), 6.65 (d, 1H), 4.25 (s, 3H), 2.51 (d, 1H), 2.25 (m, 1H), 1.35 (m, 1H), 1.25 (m, 1H), 1.05–0.75 (m, 3H), 0.65 (m, 1H), 0.52 (t, 3H); MS <i>m/z</i> 289.2 (M + 1).	0.41
37		MS <i>m/z</i> 293.2 (M + 1).	0.025
38		MS <i>m/z</i> 321.4 (M + 1).	0.74
39		MS <i>m/z</i> 333.2 (M + 1).	0.48

TABLE 3K

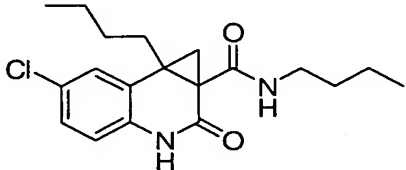
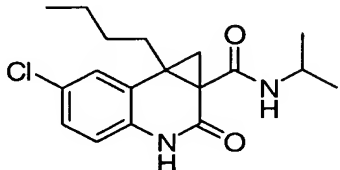
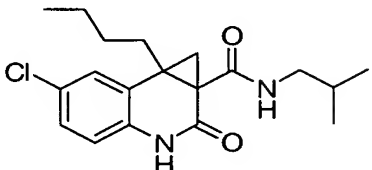
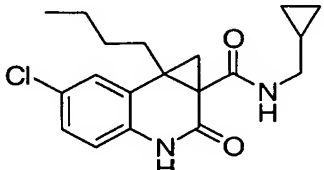
Compound Number	Structure	Physical Data ¹ H NMR 400 MHz (DMSO- <i>d</i> ₆) and/or MS (<i>m/z</i>)	EC ₅₀ (μM) (293 T)
40		MS <i>m/z</i> 349.2 (M + 1).	6.30
41		MS <i>m/z</i> 335.2 (M + 1).	2.77
42		MS <i>m/z</i> 349.4 (M + 1).	6.85
43		MS <i>m/z</i> 347.2 (M + 1).	5.69

TABLE 3L

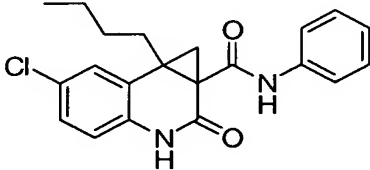
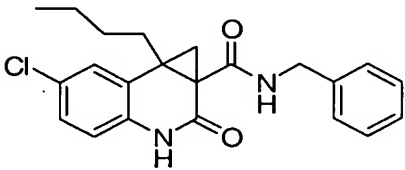
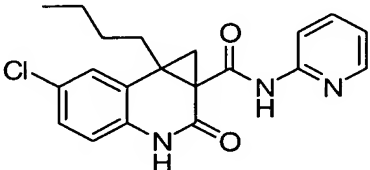
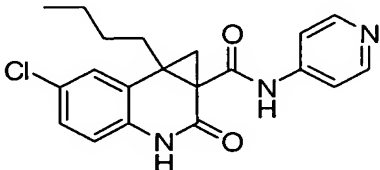
Compound Number	Structure	Physical Data ¹ H NMR 400 MHz (DMSO- <i>d</i> ₆) and/or MS (<i>m/z</i>)	EC ₅₀ (μM) (293 T)
44		MS <i>m/z</i> 369.2 (M + 1).	4.56
45		MS <i>m/z</i> 383.2 (M + 1).	2.82
46		MS <i>m/z</i> 370.2 (M + 1).	2.69
47		MS <i>m/z</i> 370.2 (M + 1).	8.25

TABLE 3M

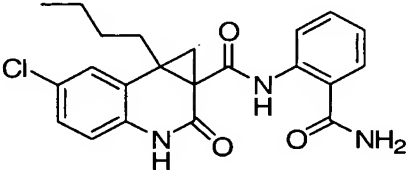
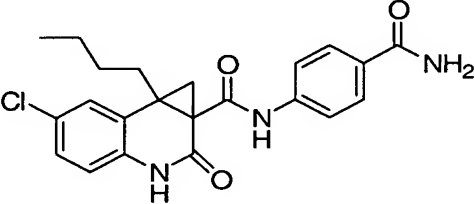
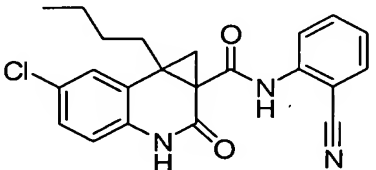
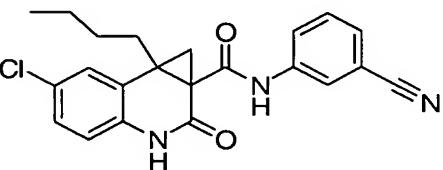
Compound Number	Structure	Physical Data ¹ H NMR 400 MHz (DMSO- <i>d</i> ₆) and/or MS (<i>m/z</i>)	EC ₅₀ (μM) (293 T)
48		MS <i>m/z</i> 412.4 (M + 1).	1.32
49		MS <i>m/z</i> 412.2 (M + 1).	4.55
50		MS <i>m/z</i> 394.3 (M + 1).	6.08
51		MS <i>m/z</i> 394.2 (M + 1).	0.16

TABLE 3N

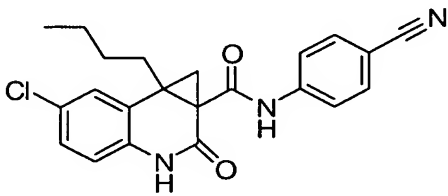
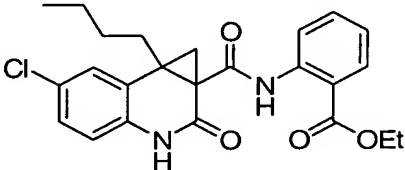
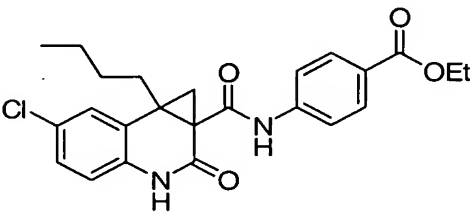
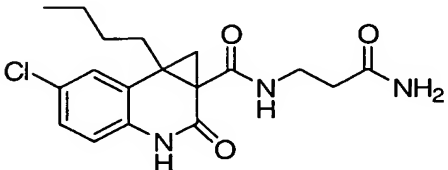
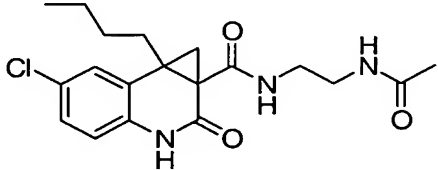
Compound Number	Structure	Physical Data ¹ H NMR 400 MHz (DMSO- <i>d</i> ₆) and/or MS (<i>m/z</i>)	EC ₅₀ (μM) (293 T)
52		¹ H NMR 400 MHz (Acetone, <i>d</i> ₆) δ 9.93 (bs, 1H), 9.32 (bs, 1H), 7.74 (dd, 2H), 7.60 (dd, 2H), 7.40 (d, 1H), 7.11 (dd, 1H), 6.92 (d, 1H), 2.43 (m, 1H), 2.14 (d, 1H), 1.39 (m, 1H), 1.31-1.14 (m, 4H), 1.02 (d, 1H), 0.58 (t, 3H); MS <i>m/z</i> 394.3 (M + 1).	NA
53		MS <i>m/z</i> 441.3 (M + 1).	6.64
54		¹ H NMR 400 MHz (Acetone, <i>d</i> ₆) δ 9.83 (s, 1H), 9.31 (s, 1H), 7.84 (dd, 2H), 7.67 (dd, 2H), 7.40 (d, 2H), 7.11 (dd, 1H), 6.92 (d, 1H), 4.18 (q, 2H), 2.46 (m, 1H), 2.14 (d, 1H), 1.40 (m, 1H), 1.35-1.05 (m, 6H), 1.00 (d, 1H), 0.59 (t, 3H); MS <i>m/z</i> 441.4 (M + 1).	8.62
55		MS <i>m/z</i> 364.4 (M + 1).	5.20

TABLE 30

Compound Number	Structure	Physical Data ¹ H NMR 400 MHz (DMSO- <i>d</i> ₆) and/or MS (<i>m/z</i>)	EC ₅₀ (μM) (293 T)
56	 <chem>CCCC12C(=O)Nc3ccc(Cl)cc3C1(CCC)C2=O</chem>	MS <i>m/z</i> 378.2 (M + 1).	NA